Apolipoprotein E Genotype is a Determinant of Low-density Lipoprotein Cholesterol and of its Response to a Low-cholesterol Diet in Type 1 Diabetic Patients with Elevated Urinary Albumin Excretion

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The effect of the apolipoprotein (apo) E genotype on the lipoprotein response to a 1 year low cholesterol diet (200 mg cholesterol per day) was evaluated in 36 patients with Type 1 diabetes mellitus with albuminuria between 10 and 200 μg min^{-1}. Apo E genotype was characterized by polymerase chain reaction and restriction isotyping. In 11 IDDM patients with at least one ϵ4 allele (apo E4 group), baseline serum total and low density lipoprotein (LDL) cholesterol were higher ($p < 0.05$ for both) than in 25 patients without an ϵ4 allele and with at least one ϵ3 allele (apo E3 group). Dietary counselling resulted in a similar decrease in cholesterol intake in both groups, whereas linoleic acid did not change. In the apo E4 group, serum total and LDL cholesterol at follow-up fell ($p < 0.01$ for both) to levels that were not different from those in the apo E3 group, and the changes in these parameters were greater ($p < 0.02$) than those in the apo E3 group. We conclude that the apo E4 allele is associated with atherogenic lipoprotein abnormalities in Type 1 DM patients with minor elevations in albuminuria when they use their habitual diet. Apo E4 carrying patients respond better to a low cholesterol diet. © 1998 John Wiley & Sons, Ltd.

Diabet. Med. 15: 1031–1035 (1998)

KEY WORDS **total cholesterol; LDL cholesterol; apo E genotype; low cholesterol diet; albuminuria; Type 1 DM**

Received 8 January 1998; revised 5 June 1998; accepted 26 June 1998

Introduction

In Type 1 (insulin-dependent) diabetes mellitus (DM) microalbuminuria is associated with increased risk of cardiovascular disease.[1,2] Unfavourable lipoprotein changes, including higher serum levels of low-density lipoprotein (LDL) cholesterol and lower levels of high-density lipoprotein (HDL) cholesterol have been demonstrated[3] and can in part explain the increased cardiovascular risk of microalbuminuria.[4]

Serum total cholesterol level is influenced by the apo E genotype, which accounts for about 8 % of cholesterol variation in the general population.[5] Three alleles, of which ϵ3 is the most common and ϵ2 is the least common, determine 6 apo E genotypes, E2/E2, E2/E3, E3/E3, E2/E4, E3/E4 and E4/E4.[5] Apo E4 carriers have the highest and apo E2 carriers the lowest LDL cholesterol concentration, with intermediate levels in apo E3 homozygotes.[5] In Type 2 DM,[6] insulin-treated,[7] and Type 1 DM patients,[8] a comparable association between apo E phenotype and LDL cholesterol levels has been found in cross-sectional studies and a recent meta-analysis indicated a higher prevalence of coronary heart disease in non-diabetic apo E4 carriers than in apo E3 homozygotes.[9] An association between the ϵ4 allele and macrovascular complications has also been shown in diabetes[6,8] but this has not been confirmed.[10]

A greater LDL cholesterol lowering effect of a low cholesterol, low fat diet has been found in non-diabetic apo E4 carriers compared to apo E3 homozygotes in several,[11–13] but not all studies.[14] The effect of dietary cholesterol on the relationship between LDL cholesterol and the ϵ4 allele in Type 1 DM is not known. Therefore, we evaluated the effect of a 1-year low cholesterol diet on the lipoprotein profile in Type 1 DM patients with and without the ϵ4 allele.

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CCC 0742–3071/98/121031–05$17.50

Methods

Patients and Design

The study was approved by the local medical ethics committee and all participants gave informed consent. Type 1 DM patients (post-glucagon C-peptide level <0.2 nmol l^{-1}) with a mean urinary albumin excretion rate (UalbV) between 10 and 200 μg min^{-1} on three consecutive overnight collections were recruited from the outpatient clinic over a 6-month period. The upper limit of normal (> the 97.5th percentile) is 10 μg min^{-1} in our clinic and such minor elevations in albuminuria have been associated with lipoprotein abnormalities[3] and progression to microalbuminuria.[15] Exclusion criteria were clinically manifest cardiovascular disease and hypertension (systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥95 mmHg). Further exclusion criteria were ketoacidosis or severe systemic diseases within 3 months prior to the study, liver disease, thyroid dysfunction, pregnancy and the use of any medication other than insulin and oral contraceptives.

The participants were divided into two groups. The apo E3 group consisted of patients with at least one ϵ3 allele and without an ϵ4 allele. The apo E4 group consisted of patients with at least one ϵ4 allele. Subjects were studied before and after a 1-year low cholesterol diet. Dietary advice included a cholesterol intake of 200 mg day^{-1}, a maximal total fat intake of 35 Energy %, a maximal saturated fat intake of 15 Energy %, and a protein intake of 15 to 20 Energy %. The diets were isoaloric for each patient. At baseline and after 1 year of follow-up a complete dietary history was obtained using the recall technique which covered a 1-week period.[16] Diet composition was assessed with a nutrient data base.[17] Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Venous blood was collected after a 10 h fast.

Laboratory Assays

Lipids were measured in whole serum and in the HDL-containing supernatant fraction after precipitation of apo B-containing lipoproteins with sodium phosphotungstate and magnesium chloride.[18] Cholesterol and triglycerides were measured by automated methods. Very low density cholesterol (VLDL) + LDL cholesterol was calculated as the difference between serum total and HDL cholesterol. LDL cholesterol was calculated with the Friedewald formula.[19] Apos AI and B were determined by immunoturbidimetery with tests from Boehringer Mannheim (Almere, The Netherlands, cat. no. 726478 and 726494, respectively).

The apo E genotype was analysed by polymerase chain reaction (PCR)-based amplification of DNA from peripheral blood leukocytes.[20] The primers spanned a 244 base-pair DNA fragment including amino acids positions 112 and 158.[20] Thirty cycles were performed using a Perkin Elmer 480 thermocycler: 60 s at 95 °C, 60 s at 60 °C and 120 s at 72 °C. Subsequently, 5 U of HhaI was added and the PCR products were digested for at least 3 h at 37 °C. The ϵ2 (112 cys, 158 cys), ϵ3 (112 cys, 158 arg) and ϵ4 (112 arg, 158 arg) alleles are shown as 91 and 81, as 91, 48 and 33, and as 72, 48 and 33 base-pair bands, respectively. Polyacrylamide gel electrophoresis was used to separate restriction fragments, followed by ethidium bromide staining and visualization on an ultraviolet transilluminator. Urinary albumin was measured by radioimmunoassay (cat. no. KHAD2, Diagnostics Products Corp., Apeldoorn, The Netherlands). Haemoglobin A_{1c} (HbA_{1c}) was measured by high performance liquid chromatography after removal of the labile fraction (Bio-Rad, Veenendaal, The Netherlands; normal range 4.6 to 6.1 %).

Statistical Analysis

Data are given in mean ± SD and in geometric means (95 % confidence intervals). Between group differences in (changes in) parameters were compared by the Mann-Whitney test. Changes in parameters from baseline in each group were evaluated by the paired Wilcoxon test. Spearman's rank correlation coefficients (r_s) were used to determine bivariate relationships between variables. Multiple regression analysis was used to assess the independent relationships between parameters. A two-sided p value <0.05 was considered significant.

Results

Thirty-six Type 1 DM patients participated. In the apo E3 group, 23 patients were apo E3 homozygotes and two were apo E2/E3 heterozygotes. The apo E4 group consisted of 11 patients, 8 apo E3/E4 heterozygotes, 2 apo E4 homozygotes, and 1 apo E2/E4 heterozygote. The overall frequencies of the ϵ2, ϵ3 and ϵ4 alleles were 4 %, 78 %, and 18 %, respectively, and the apo E genotypes were in Hardy-Weinberg equilibrium (χ^2 = 1.59, df = 5, $p > 0.80$). Since the frequency of the ϵ2 allele was expectedly low and it was similarly distributed among the two groups, the presence of the ϵ2 allele was not considered further.

Table 1 shows the clinical parameters of the two groups at baseline. In the apo E3 group one woman used oral contraceptives and one woman was postmenopausal. Two of the three women in the apo E4 group were postmenopausal. At follow-up there were no significant changes in HbA_{1c} and insulin dose. BMI and UalbV increased slightly in the apo E3 group, but were not different between the groups at follow-up.

At baseline, total and saturated fat intake were higher in the apo E3 group without differences in other dietary parameters (Table 1). After 1 year of follow-up cholesterol intake decreased similarly in both groups. In the apo E4 group, no further changes in diet composition were noted. In the apo E3 group, total and saturated fat intake

Table 1. Clinical characteristics and diet composition of 25 Type 1 DM patients without (apo E3 group) and 11 with at least one apolipoprotein ϵ4 allele (apo E4 group)

	Apo E3 group (n = 25)	Apo E4 group (n = 11)
Age (years)	42 ± 12	43 ± 10
Diabetes duration (years)	23 ± 10	23 ± 14
Gender (F/M)	3/22	3/8
Body mass index (kg m^{-2})		
Baseline	23.9 ± 2.5	25.3 ± 2.2
Follow-up	24.3 ± 2.8[a]	25.4 ± 2.7
HbA_{1c} (%)		
Baseline	7.9 ± 0.9	8.2 ± 1.1
Follow-up	8.0 ± 1.0	8.1 ± 1.0
Smokers (n)	11	6
Urinary albumin excretion rate (μg min^{-1})		
Baseline	33 (22–49)	30 (18–51)
Follow-up	45 (27–74)[a]	40 (15–108)
Insulin dose (U day^{-1})		
Baseline	60 ± 22	61 ± 14
Follow-up	55 ± 21	62 ± 13
Energy (MJ day^{-1})		
Baseline	9.2 ± 2.4	8.1 ± 2.4
Follow-up	8.4 ± 2.4	7.9 ± 2.3
Cholesterol (mg day^{-1})		
Baseline	285 ± 118	283 ± 91
Follow-up	198 ± 102[c]	204 ± 94[b]
Total fat (Energy %)		
Baseline	42 ± 5[d]	37 ± 7
Follow-up	38 ± 5[a]	36 ± 4
Saturated fat (Energy %)		
Baseline	17 ± 3[d]	14 ± 3
Follow-up	14 ± 4[b]	14 ± 4
Linoleic acid (Energy %)		
Baseline	7 ± 4	7 ± 3
Follow-up	8 ± 4	7 ± 4

Data in mean ± SD and geometric mean (95% confidence intervals).
[a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$ from baseline, [d]$p < 0.02$ from apo E4 group.

decreased, but no differences in diet composition were demonstrated between the groups at follow-up.

At baseline mean serum levels of total cholesterol, VLDL + LDL cholesterol, LDL cholesterol and apo B were higher in the apo E4 than in the apo E3 group (Table 2), with no differences in serum triglycerides, HDL cholesterol or apo AI levels between the groups. In the apo E4 group, serum total cholesterol, VLDL + LDL cholesterol, LDL cholesterol and apo B decreased at follow-up to levels that were not different from those in the apo E3 group. These decreases were greater than the insignificant changes in the apo E3 group (Table 2). HDL cholesterol decreased in the apo E3 group, but its change was not different ($p > 0.80$) from the change ($p = 0.11$) in the apo E4 group. Apo AI levels did not change in either group.

The changes in LDL cholesterol at follow-up were related to the changes in cholesterol intake (in mg kg^{-1} $bodyweight^{-1}$) in the apo E4 group ($r_s = 0.64$, $p < 0.05$) but not in the apo E3 group ($r_s=0.25$, $p = 0.23$). In the whole study population, the changes in LDL cholesterol were correlated with the individual changes in linoleic acid intake ($r_s = -0.32$, $p < 0.05$).

In the apo E4 group, multiple regression analysis showed that the relationship between changes in LDL cholesterol at follow-up and changes in cholesterol intake ($r = 0.64$, $p = 0.034$) was independent from changes in linoleic acid intake ($p = 0.59$) and baseline LDL cholesterol ($p = 0.38$).

Discussion

This study demonstrates that the apo E4 allele is associated with higher levels of serum total cholesterol, LDL cholesterol, and apo B in selected Type 1 DM patients when they consume their habitual diet. Furthermore, LDL cholesterol significantly decreased in response to a low cholesterol diet in apo E4 carriers only, in whom its change was related to the change in cholesterol intake. These observations support the notion that the LDL

Table 2. Plasma (apo)lipoproteins in 25 Type 1 DM patients without (apo E3 group) and with at least one apolipoprotein ϵ4 allele (apo E4 group)

	Apo E3 group (n = 25)	Apo E4 group (n = 11)
Total cholesterol (mmol l^{-1})		
Baseline	5.86 ± 1.16	6.88 ± 0.97[b]
Follow-up	5.66 ± 1.02	5.98 ± 1.27[c,e]
VLDL + LDL cholesterol (mmol l^{-1})		
Baseline	4.47 ± 1.17	5.44 ± 1.02[a]
Follow-up	4.41 ± 0.95	4.64 ± 1.28[c,e]
LDL cholesterol (mmol l^{-1})		
Baseline	3.91 ± 1.02	4.83 ± 0.98[b]
Follow-up	3.85 ± 0.95	4.16 ± 1.26[c,d]
HDL cholesterol (mmol l^{-1})		
Baseline	1.39 ± 0.33	1.45 ± 0.28
Follow-up	1.25 ± 0.35[c]	1.35 ± 0.35
Triglycerides (mmol l^{-1})		
Baseline	1.01 (0.81–1.26)	1.20 (0.86–1.68)
Follow-up	1.03 (0.81–1.31)	0.99 (0.76–1.28)
Apolipoprotein B (g l^{-1})		
Baseline	0.86 ± 0.24	1.03 ± 0.22[a]
Follow-up	0.87 ± 0.21	0.86 ± 0.18[c,e]
Apolipoprotein AI (g l^{-1})		
Baseline	1.73 ± 0.30	1.78 ± 0.23
Follow-up	1.77 ± 0.26	1.74 ± 0.36

Data in mean ± SD and geometric mean (95% confidence intervals).
[a]$p < 0.05$ and [b]$p < 0.02$ from apo E3 group; [c]$p < 0.01$ from baseline; [d]$p < 0.02$ and [e]$p < 0.01$ from change in apo E3 group.

cholesterol-raising effect of the ϵ4 allele in Type 1 DM is mediated at least in part by dietary cholesterol.

The higher serum cholesterol level in apo E4 carrying Type 1 DM patients raises the possibility that the apo E genotype influences the high cardiovascular risk in such patients. In the Dutch population, the ϵ4 allele frequency is 14 to 17 %[21] and was 18 % in the current study, similar to that reported in microalbuminuric Caucasian Type 1 DM patients from the USA.[22] In that study the prevalence of the ϵ4 allele did not significantly vary with the presence of (incipient) nephropathy,[22] making an important association between apo E genotype and diabetic renal involvement unlikely.

The habitual diet composition of our patients resembled that of the general Dutch population.[23] A reduction in cholesterol intake was the main aim of dietary counselling. Little change occurred in dietary fat content and composition, reducing the possible confounding effects of such intervention on LDL cholesterol. Nevertheless, changes in LDL cholesterol were correlated with individual changes in linoleic intake, in keeping with an LDL cholesterol lowering effect of linoleic acid in Type 1 DM.[25] The effect of a reduction in dietary cholesterol on LDL cholesterol is highly variable.[24] Its slight fall in the apo E3 group approximated the expected effect for the general population,[24] but the change was considerably larger in the apo E4 group. Bias due to regression to the mean could have contributed to this. However, the correlation between changes in dietary cholesterol intake and changes in LDL cholesterol in the apo E4 group was independent of the individual changes in linoleic acid intake as well as from baseline LDL cholesterol, supporting our conclusion that the ϵ4 allele is a determinant of the LDL cholesterol response to a low cholesterol diet in Type 1 DM.

Among possible mechanism for the LDL cholesterol raising effect of the ϵ4 allele, an enhancement of dietary cholesterol absorption efficacy in apo E4 carriers compared to apo E3 homozygotes has been shown.[26] This would promote hepatic cholesterol accumulation, lower hepatic LDL receptor expression and a higher LDL cholesterol level.[13,26]

Apo E is subject to post-translational modification.[27] Phenotyping techniques like isoelectric focusing may lead to misclassification of an apo E isoform in as much as 17 to 24 % of cases.[27] We circumvented this analytical problem by apo E genotyping. The use of the Friedewald formula was used to calculate LDL cholesterol, and precipitation of apo B-containing lipoproteins to measure HDL cholesterol may overestimate LDL cholesterol compared to ultracentrifugation analysis.[28,29] To avoid possible effects of high triglycerides on the calculated LDL cholesterol level, we also evaluated the influence of the ϵ4 allele on VLDL + LDL cholesterol. This comparison, as well of that of apo B, matched the observed effects of the ϵ4 allele on LDL cholesterol.

In conclusion, this study shows that part of the variation in LDL cholesterol levels in Type 1 DM patients with elevated urinary albumin excretion can be explained by the apo E genotype. Apo E4 carrying Type 1 DM patients

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respond better to a low cholesterol diet than patients without the allele, and the former may benefit most from dietary counselling.

Acknowledgements

The technical assistance of J Brouwer and B.K. Stulp in apo E genotyping is appreciated. K. Hoogenberg is supported by grant from the Dutch Diabetes Foundation.

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